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1. A.I.C. by examination is recognised as equivalent to M.Sc. degree in chemistry for purposes of recruitment of chemists as per G.O. No. F. 18—36/57-T-5, dated 19-8-58 of the Ministry of Scientific Research and Cultural Affairs. The examination with Section 2 (Drugs and Pharmaceuticals) is duly recognised in the Drugs Rules, Government of India. The nineteenth Examination is expected to be held at Calcutta in or around November, 1969, and will comprise of Theoretical, Practical and Oral tests.

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Sub-Group	*Section*	*Subject*
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	3.	Analysis of Foods.
	9.	Analysis connected with Forensic Chemistry.
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II	2.	Analysis of Drugs and Pharmaceuticals.
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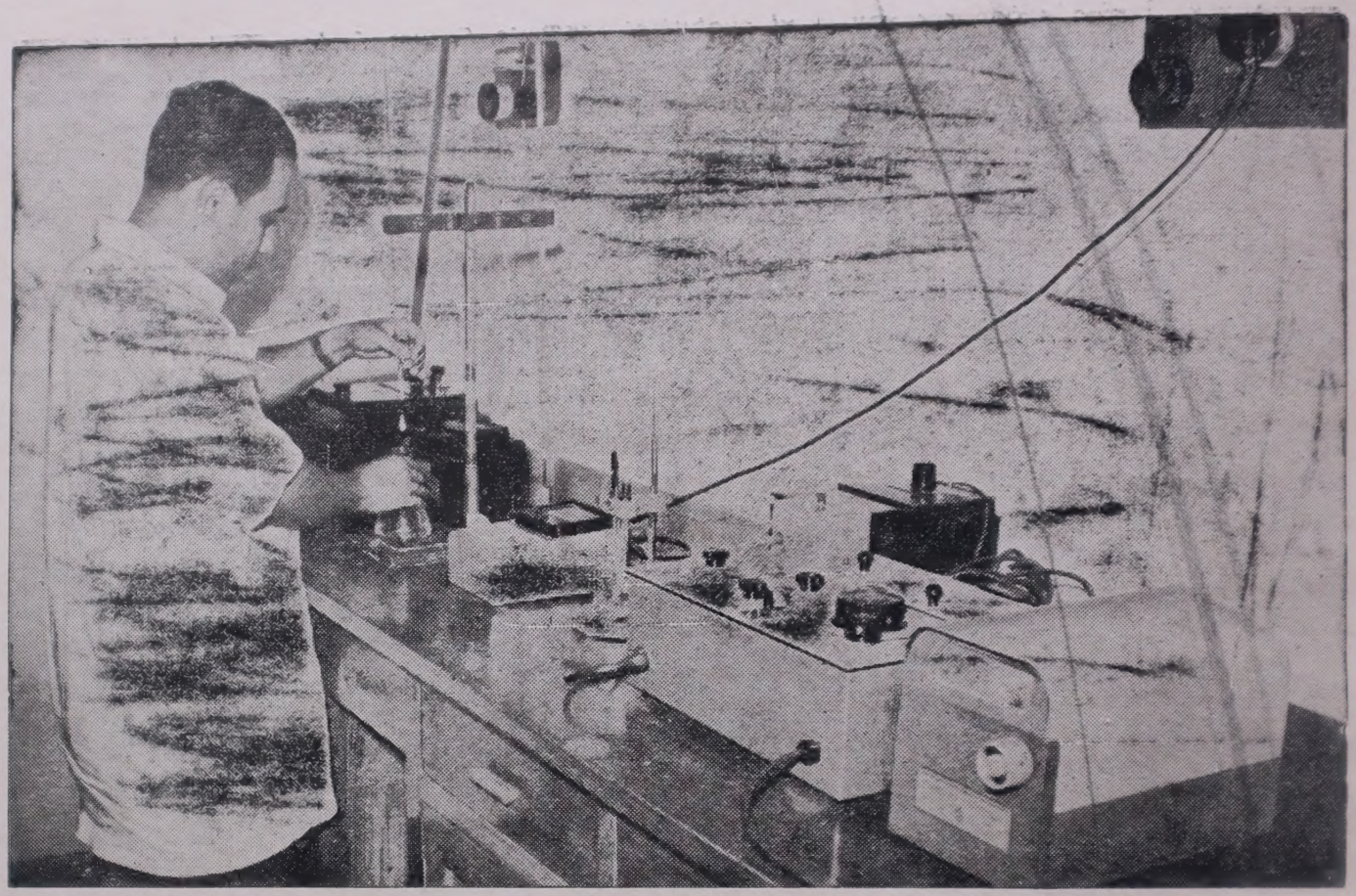

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VOL. XI MARCH, 1968 PART II

CONTENTS

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The manuscript should be submitted in duplicate. It should be neatly typed with double line and marginal spacings in the final form for printing according to the usual get up of this Journal. No substantial alteration is allowed at the proof stage. Foot-notes should be avoided as far as possible, tables and figures minimum in number and size and should not represent the same data. Tables and figures should be numbered consecutively and drawings in black ink. References, numbered consecutively, should be at the end of the paper. In the text these should be indicated by superior numbers (above the line).

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Vol. XL MARCH, 1968 Part II

FOOD POISONING

In the past, most food poisoning cases could be classified under copper poisoning due to cooking in copper vessels with fat or oil, or argemone poisoning due to ingestion of oil contaminated with alkaloids of seeds of *Argemone mexicana*, which look like black mustard seeds. There were some sporadic cases of divergent and often unknown etiology particularly those for homicidal purposes, in addition to cases due to bacterial contamination of foods. Inspite of strict vigilance, argemone poisoning, instead of dying out, has spread its domain of contamination from oils to *vanaspati* (hydrogenated oil). Because of advancement of science and industry, newer items are coming up, e.g. folidol and trieresyl phosphate. Folidol contamination is mostly due to improper use by illiterate people. During a field investigation, the writer learnt from the head of an affected family of farmers that they *always* destroy the empty containers, while a little boy suddenly appeared with a bright, empty aluminium bottle of folidol. It is difficult to believe that poor farmers can give up the temptation of using such empty containers.

Though Malda paralysis as it took place in Malda in West Bengal and Darrang in Assam did not have anything to do with empty containers of trieresyl phosphate, use of such containers for storage of edible oils was responsible for most outbreaks of the paralytic disease even in the more advanced countries. An outbreak of this disease on a small scale has recently been observed in Kankinara area in West Bengal through contamination of mustard oil with trieresyl phosphate. It is most important for the authorities concerned to take effective measures for proper disposal of the empty containers and to stop contamination of foods with such highly toxic materials.

For some time past fatal liquor poisoning cases in large numbers are being reported mostly in and around Calcutta. Apart from some guess regarding contamination with methylated spirit nothing has come out with regard to the real toxic agent. Whereas checking of foods is looked after by Health Departments and non-alcoholic bevarages are accepted as items of food, alcoholic liquors are not included under foods. It is high time that such liquors should also be included in Prevention of Food Adulteration Rules, and such liquor poisoning cases should be investigated by Health Departments concerned as these are seriously connected with health.

R. N. C.

J. Inst. Chemists (India), Vol. XL, March, 1968

STRYCHNINE GROUP—PART I

By

R. N. Chakravarti

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General Introduction

A number of plants of the genus Strychnos (Family, Loganiaceae) contains a group of closely related alkaloids, some of which have got strong physiological action. These alkaloids may be divided into three subgroups:

(a) Strychnine, brucine, α-colubrine, β-colubrine, novacine, vomicine,
(b) Strychnospermine, spermostrychnine,
(c) Calabash curare alkaloids.

Among the strychnos alkaloids, strychnine is evidently the most important in almost every respect. It is available in large amounts from plants, obtained in the pure state with comparative ease and is much more in use. 'For its molecular size it is the most complex substance known'.[1] In view of this it drew the closest attention of many distinguished chemists and a huge volume of highly critical work was carried out. The problem of the structure of strychnine was solved by purely classical methods involving preparation of a number of highly interesting derivatives and laborious stepwise degradations with appropriate interpretations. It is quite significant to point out that the problem could be solved finally just before the advent of the modern era when classical methods are going out of vogue in the face of more convenient physical methods. In view of the application of the classical methods, however, a wealth of information is available which would, otherwise, have been missed. It is significant to mention in this connection that the mass spectrum of strychnine[2] shows a pronounced molecular ion and hardly any helpful fragmentation which is essential for determination of the structure.

For reasons stated above, in the present account on the strychnine group, the case of strychnine is dealt with in comparatively greater detail. This will enable one to have an idea about the nature of the problems involved in this group of alkaloids and the procedure adopted for their solution.

Strychnos Sp.

Strychnine and brucine are present in a number of plants of the genus *Strychnos*[3], some of which, however, contain curare group of alkaloids. These are present in plants used mostly as arrow poisons. *Strychnos nux vomica* Linn., or poison nut, is a deciduous tree, about 40 feet in height, growing throughout

south-eastern Asia and in northern Australia. The principal country of origin is India. The ripe fruit looks like an orange, though much smaller in size, and contains a number of hard, extremely bitter button like seeds. Strychnine and brucine are chiefly obtained from these seeds. The alkaloids are also present in bark, root, leaves, the hard fruit shells and the wood. The seeds contain 2 to 3 per cent of total alkaloids of which about 50 per cent is strychnine and major portion of the remainder is brucine. Various storage conditions of the seeds were studied by Puntambekar and Krishna[4]. *Strychnos Ignatii* Berg. is a large climbing shrub growing mainly in the Philippines. From the fruits of this plant are derived 'St. Ignatius' beans. The beans contain 2 to 3 per cent of total alkaloids of which about 65 per cent is strychnine and the remainder is mainly brucine. The red branch bark of *S. icaja* of Congo has been reported[5] to contain as high as 15·9% of total alkaloids, over 40% of which is strychnine.

Strychnine, Brucine, α- and β-Colubrines

Isolation

Pelletier and Caventou isolated strychnine in 1817 and brucine in 1819 from *Strychnos nux vomica* Linn. (cf. Robinson)[6]. In view of the industrial importance of strychnine, considerable work was carried out[7-11] on the process of isolation of the alkaloids and separation of strychnine and brucine. A convenient method consists in making a paste of the powdered nux vomica seeds with water and lime, followed by drying and extraction with chloroform or benzene. The total alkaloids are then separated from the extract with dilute sulphuric acid and precipitated with ammonia or caustic soda. From the precipitate strychnine is obtained by fractional crystallization from aqueous alcohol of appropriate strength. Brucine sulfate crystallizes out on neutralization of the mother liquor with sulfuric acid. α- and β-colubrines, and pseudostrychnine (16-hydroxystrychnine) were isolated by Warnat[12] from the mother liquors in the manufacture of strychnine. Vomicine was also isolated from mother liquors of strychnine manufacture[13]. According to Warnat 'strychnicine' of van Boorsma[14] (1902) and 'struxine' of Schaefer[15] are not single entities. 'Igasurin' of Desnoix[16] was also found to be a mixture of alkaloids[17]. More recently novacine, a minor alkaloid, has been isolated from nux vomica seeds. This has been identified with N-methyl-sec-pseudobrucine[18].

General Properties, Tests and Estimations

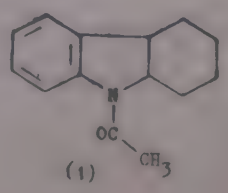
Strychnine : Strychnine crystallizes in colorless rhombic prisms from alcohol, m.p. 266° to 290° depending considerably on the rate of heating—a difficulty often experienced in this group, b.p. 270°/5 mm., $[\alpha]^{20}_{D}$ −104° (alcohol), $[\alpha]^{18}_{D}$ −139·3° (chloroform). It is almost insoluble in ether, very slightly soluble in water, but readily soluble in chloroform or boiling 90% alcohol. It is extremely bitter. Its bitterness is perceptible even at a dilution of 1 in 700,000 in water. Strychnine is used in medicine as a bitter tonic and as a stimulant. In larger doses it is highly toxic. Since the original finding of Magendie[19] in this respect, considerable work has been carried out on the pharmacological action of the alkaloid. 30 to 60 mg. is said to be fatal to man.

The poisoning is manifested by painful tetanic spasms involving all the muscles of the body, paralysis of the central nervous system and finally respiratory failure.

Brucine, α- and β-Colubrines : Brucine crystallizes from water or weak aqueous alcohol in monoclinic prisms with $4H_2O$, m.p. 105°, or from alcohol with $2H_2O$, m.p. of anhydrous product 178°, $[\alpha]^{20}$ −121° (chloroform). In most of the solvents its solubility is greater than that of strychnine. Brucine is also extremely bitter but its toxic effect is much less. α-Colubrine is obtained from hot dilute alcohol in white shining crystals with $4H_2O$, m.p. 184° (anhydrous), $[\alpha]^{19}_D$-76·5° (80% alcohol). β-Colubrine is obtained anhydrous from dilute alcohol, m.p. 222°, $[\alpha]^{19}_D$-107·7° (80% alcohol). The colubrines are easily soluble in alcohol, benzene and chloroform.

Salts : Strychnine forms a large number of well-defined salts, e.g. $B.HNO_3$ (needles), $B.HCl.2H_2O$ (efflorescent trimetric prisms), $B.HBr.H_2O$, $B.HI.H_2O$, $B_2.H_2SO_4.5H_2O$ (prisms) (also $6H_2O$). The salts of brucine, e.g. B.HCl, B.HBr, $B_2H_2SO_4.7H_2O$ (needles), are usually much more soluble in water than corresponding salts of strychnine, but reverse is true for the hydriodides[20]. Salts of α-colubrine, e.g. $B.HCl.3H_2O$ (leaflets), $B_2.H_2SO_4.10H_2O$, and of β-colubrine, e.g. $B.HCl.H_2O$ (leaflets), $B_2.H_2SO_4.9H_2O$ (prisms) are easily soluble in water.

Color Reactions, Estimation : Strychnine is one of the most stable alkaloids. It is not colored by sulfuric acid even at 100°. With nitric acid it gives only a weak yellow color. When a small quantity of strychnine in sulfuric acid is treated with a minute quantity of an oxidizing agent ($K_2Cr_2O_7$, MnO_2, $KMnO_4$ etc.) an immediate blue coloration is produced, which changes into violet, red and finally yellow[21]. The 'Otto reaction' is carried out by adding a minute quantity of potassium dichromate to the base in 80% sulfuric acid. A fine reddish purple coloration indicates presence of strychnine. Derivatives of strychnine having the hexahydrocarbazole moiety e.g. N-acetylhexahydrocarbazole (1)[22] also respond to this test. Strychnine can also be tested by the 'strychnidine reaction'. When it is briskly boiled with excess of amalgamated zinc dust and concentrated hydrochloric acid so that most of the acid is neutralized, the diluted filtered solution develops a bright red color with ferric chloride or sodium nitrite.[23] When brucine is treated in sulfuric acid solution with oxidizing agents like chromic acid only a deep red coloration is produced. The deep red coloration is also produced when a solution of brucine is treated with nitric acid. This coloration provides a characteristic test for either of the reactants. When stannous chloride or other suitable reducing agent is added cautiously to the red solution, the color changes to violet. Brucine may thus be differentiated from other substances which give a red coloration with nitric acid.[24]



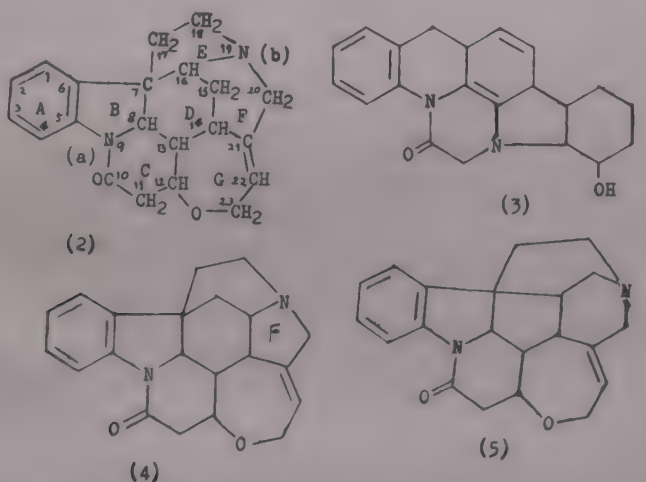
(1)

Methods are available for estimation of strychnine in pharmaceutical preparations.[27-29] It is said to be convenient to decompose brucine with nitric acid and then carry out the estimation of strychnine if it is contaminated with the former. When a solution of the salts of the two bases is made sufficiently acidic and treated with potassium ferrocyanide, strychnine ferrocyanide is precipitated. From the ferrocyanides, the individual bases may be regenerated with ammonia. It may be utilized either as a gravimetric or as a volumetric method.[30-31]. Chromatographic methods for separation and estimation of strychnine and brucine are also available.[32-33]

Structure

Introduction :

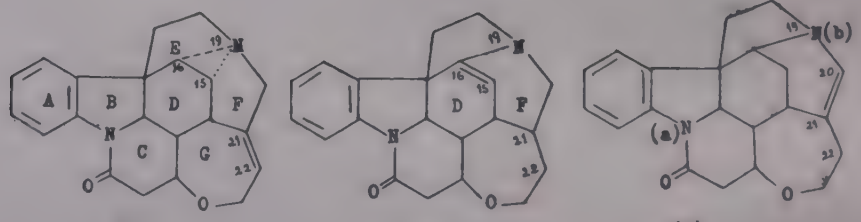
After the isolation of strychnine in 1817, its molecular formula was established as $C_{21}H_{22}O_2N_2$ by Regnault[34] in 1838. Although Loebish and Schoop made some contribution in this respect around 1885, Tafel[35] may be described as the pioneer worker with regard to serious chemical investigation in this field. His contribution included proof for the occurrence of a benzene ring, a tertiary basic nitrogen and an amide group, and thus supplied the fundamental basis for building up the structure of this most complicated and highly interesting alkaloid which was ultimately established as (2).[36-39]



Leuchs and his collaborators started on their project around 1908.[40] Unlike the later workers in this field, a major part of his research activity was concentrated in this particular line only and as a result about one hundred and twentyfive memoirs, comprising of fruits of long, tenacious and hard labor, came out from his laboratory. He had about sixty collaborators in his venture covering well over thirtyfive years of active investigation. He was the first to undertake well-planned stepwise degradation, mostly oxidative in nature, although going through his earlier papers one has to be satisfied with fragments of the structure only.

With the entry of Robinson, in 1909, into this field the investigation definitely took a sharp turn for the complete structure. His zeal for having a working hypothesis for the complete structure was evident even in his earliest paper on the subject in which (3) was placed as the structure of strychnine.[41] In his earlier papers he was associated with Perkin. Considering the problem as a whole, his contribution may be described as most intelligent and most distinguished. In this respect, it is equally significant to point out that the final structure (2) for strychnine, which was firmly established beyond doubt, also came out from the pen of this versatile chemist.[36]

The investigation on the structure of strychnine had an extremely exciting finish during 1945-1947. The situation at times became quite tense and thrilling. In 1932, the structure (4) was put forward by Menon and Robinson[42] for strychnine. It may be described as the penultimate structure. After thirteen years of survival without a challenge, its validity was questioned by Prelong and Szpilfogel[43-44] based on experimental evidences, when it was pointed out that the ring F in strychnine is six-membered, and not five-membered as depicted in (4). The structure (5) was put forward by these authors in place of (4). Although structure (5) did not receive any support, the six-membered ring F was accepted by all. To incorporate the six-membered ring F in the penultimate structure (4), Robinson[36] ingeniously shifted the bond connecting ring D with the basic nitrogen, N(b) at 19-position, from the 15- to 16-position (as in 6) and arrived at the final structure (2) for strychnine with a five-membered ring E.



(6) (7) (8)

The chapter could not be closed, however, inspite of this, as difficulties arose in ascribing a stable structure to neostrychnine, which, according to the notion prevailing at the time should have a double bond in the 15 : 16-position of (2) in place of the double bond of strychnine at the 21 : 22-position and should be represented as (7). This latter structure does not at all represent a stable form in view of the considerable strain involved in rings D/F in incorporating the double bond at the bridge-head 15 : 16-position[45], and it is quite unreasonable to expect the strychnine double bond at the 21 : 22-position to jump so many steps to such an unfavorable position during the conversion of strychnine into neostrychnine, which proceeds in practically quantitative yield with Raney nickel in refluxing xylene (Chakravarti and Robinson[46]). This difficulty reflected serious suspicion even on the structure (2) for strychnine. The situation became quite tense at this stage. The urgency for a detailed

and critical study of the chemistry of the neobases became evident and was taken up in all earnestness by Chakravarti and Robinson. The first obstacle in this respect, that of having sufficient quantity of neostrychnine, was solved by the discovery of the Raney nickel method. With the unravelling of the chemistry of neostrychnine by them[39] it was possible to locate the double bond of neostrychnine at the 20 : 21-position (as in 8), which is just adjacent to the strychnine double bond (cf. 2). The only challenge to structure (2) for strychnine, as provided by the difficult position of the double bond of neostrychnine, having thus been overcome it was possible to establish structure (2) for strychnine beyond any doubt whatsoever (Chakravarti and Robinson[39, 47]). An immense volume of interesting results were obtained on the Hofmann and Emde degradations of strychnine and its derivatives but for various reasons these are not included here.

Further confirmation of structure (2) was soon available from analysis of X-ray diffraction data and this valuable work also established the stereochemistry of strychnine.[48-51] The Himalayan task involved in the total synthesis of strychnine was accomplished by Woodward and his collaborators[52] starting from 2-veratrylindole. This may be unequivocally described as the greatest achievement of the period in the synthesis of natural products. The intricacies of ingenious planning, that took place behind the screen at each step of this gigantic multistage achievement, have been presented in a valuable memoir by Woodward,[53] *'modus operandi'* as he calls it. It makes very pleasant and useful study particularly for its lucid exposition and literary value. Important contribution was also made by Woodward[54] with regard to the biogenesis of strychnine.

A comprehensive account of the position prevailing around 1930 was presented by Robinson in his famous Bakerian Lecture[6] of the Royal Society, London. His more recent reviews[1, 23] on the subject are highly rational and informative though presented in rather condensed forms. These should be read by all interested in the subject.

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NOTE ON THE USE OF DIRECT COMPLEXOMETRIC TITRATION IN THE ANALYSIS OF ANTACID TABLET CONTAINING MAGNESIUM TRISILICATE I. P. HEAVY MAGNESIUM CARBONATE I. P., DRIED ALUMINIUM HYDROXIDE GEL. I. P. DRIED ALUMINIUM GLYCINATE N. F.

By

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The antacid tablet with aforesaid constituents can be estimated by the conventional method of precipitation by (a) ammonium hydroxide and ammonium chloride or (b) 8-hydroxyguinoline from ammoniacal solution or acetic acid solution buffered with acetate (c) or as phosphate and then ignited. But aluminium can be precipitated in the absence of calcium and magnesium from hot solution to help better coagulation. Excess of ammonium hydroxide dissolves the hydroxides formed. But as magnesium is present these methods are not satisfactory. There is a tendency of aluminium oxinate to absorb oxine in excess. Magnesium can be estimated as magnesium pyrophosphate. Magnesium is precipitated as Magnesium ammonium phosphate from ammoniacal solution by ammonium phosphate in absence of aluminium because Al. is also precipitated as phosphate. A long period is required for precipitation of magnesium in absence of any metal. The condition should be such that only precipitate of Mg. NH_4 PO_4, $6H_2O$ is produced, there should be no precipitate of Mg_3PO_4, $Mg(NH_4)_4(PO_4)_2$ at all. The conventional method is troublesome and time consuming, so the present communication advocates the simple and time saving complexometric titration using xylenol organge and solochrome black as indicator.

REAGENTS

(1) $\frac{M}{20}$ EDTA-18·61 gms of $C_{10}H_{14}N_2Na_2O_8 \cdot 2H_2O$ is dissolved in 1 litre of water.

(2) $\frac{M}{20}$ $PbNO_3$—16·56 gms. of $PbNO_3$ is dissolved in 1 litre of water.

(3) Solochrome black indicator- 0·5 gm of solochrome black WDFA and 4·5 gms of hydroxylamine hydrochloride an dissolved in 100·0 ml. of ethyl alcohol.

(4) Xylenol orange- 0·1 gm. of xylenol orange is dissolved in 100·0 ml of distilled water.

(5) Ammonia-Ammonium chloride, strong solution- 67·5 gms of NH_4cl is dissolved in 650·0 ml of strong ammonium hydroxide and sufficient water is added to produce 1000·0 ml.

(6) Congored paper-unglazed paper is impregnated in 0·1% $\frac{W}{W}$ solution of congored in alcohol (95%).

(7) Sodium hydroxide solution—4% solution of sodium hydroxide A.R. in water is prepared.

(8) Concentrated Hydrochloric acid A.R.

(9) Hexamine B.P.

(10) Triethanolamine.

Procedure

The average weight per tablets is determined. The tablets are powdered. About 1·0 gm. of the powder is weighed accurately in a beaker. To it, 7 ml. of conc. HCl is added ; the beaker is heated on a water bath avoiding any charring or spurting of the material. Finally the acid is evaporated to dryness and 50 ml. of water is added to the content. The residue is washed several times with warm water and the volume of 250 ml. is produced after filtration. The residue is dried and then ignited in a tared platinum crucible at 1100°C. The crucible is cooled and the residue is weighed as crude SiO_2. The residue in crucible is moistened with a little HF and H_2SO_4, dried and ignited to constant weight at 1100°C. The differences in the two weights is the weight of pure silica. The content of magnesium trisilicate is calculated as weight of pure silica multiplied by 2·165.

The filtrate of 250 ml. volume contains solution of aluminium chlorides and magnesium chloride. Aluminium chloride is calculated as Al_2O_3 using complexometric titrations with xylenol orange as metal Indicator. 25 ml. of the filtrate is transferred in a beaker. The acid is neutralised by 4% NaOH solution using congored paper as indicator. 50 ml. of $\frac{M}{20}$ EDTA solution is added to it and the solution is warmed on water bath for 30 minutes. It is cooled and 3 gms. of hexamine, 0·4 ml. of xylenolorange as indicator are added to it. The solution is titrated with $\frac{M}{20}$ $PbNO_3$ solution from pale yellow to orange colour. A blank is performed. The difference in the two titrations represents the actual amount of $\frac{M}{20}$ EDTA solution consumed by the metal Al. Each ml. of $\frac{M}{20}$ EDTA solution used up is equivalent to 0·002549 gm. of Al_2O_3.

Total Magnesium present in the tablet can be estimated as MgO by the complexometric titration with solochrome black solution as indicator.

25 ml. of the filtrate is taken in a conical flask. 10 ml. of strong NH_4OH-NH_4Cl solution, 3 ml. of triethanolamine and 0·2 ml. of solochrome black indicator are added to it. The solution is titrated with $\frac{M}{20}$ EDTA from wine red to clear blue colour. A blank is simultaneously done, the difference in the two filtrations represents the real amount of $\frac{M}{20}$ EDTA used up by total MgO. Each ml. of $\frac{M}{20}$ EDTA required is equivalent to 0·002016 gm. of MgO. From the total MgO found the quantity of MgO equivalent to that of Magnesium Trisilicate as obtained by determination of SiO_2 is subtracted. The balance MgO will give the figure of MgO coming from magnesium carbonate (heavy). The content of magnesium carbonate is calculated as (Balance MgO) multiplied by 2·381.

Discussion

There is no standard method of estimating MgO in magnesium carbonate but the residue on ignition is 42 to 45%, magnesium trisilicate contains 30·0 to 32·5 % of MgO and 66·0 to 69·5% of SiO_2. Again the National Formulary states the method of estimating Basic Aluminium Glycinate as Al_2O_3 by ignition of $Al(OH)_3$, but the ignited Al_2O_3 is very hygroscopic and therefore difficult to weigh speedily, and the result obtained by this method is a bit erroneous. The direct complexometric titrations provided a simple and quick method of estimation of the stated amounts of the constituents of the tablet with reasonable accuracy.

Acknowledgement

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STABILITIES OF BIVALENT METAL CHELATES OF N-BENZOYL-N-O-TOLYLHYDROXYLAMINE

By

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N-Benzoyl-N-o-Phenylhydroxylamine (BPHA) has been introduced by Shome[1] as an improvement over Cupferron. It has found extensive analytical applications.[2-5] Substitution of different groups at different positions of the aryl nuclei of the parent compound influenced the stabilities of resulting metal chelates. The present investigation deals with the determination of stabilities of bivalent metal chelates of ortho methyl substituted benzoylphenylhydroxylamine, by employing Bjerrum-Calvin pH titration technique[6]. Titrations were carried out in duplicate using 40 : 1 ratio of chelating agents to metal ion concentration.

EXPERIMENTAL

Ligand solution : N-benzoyl-N-o-tolylhydroxylamine was prepared by employing the method given by A. K. Majumdar and G. Dass[7]. A weighed amount of this compound was dissolved in 70 : 30 v/v dioxan-water mixture and diluted to give a 0·04 M solution.

Standard palladium, copper, nickel, Zinc and manganese solutions :

Analytical grades of palladium chloride, copper chloride, nickel sulphate, zinc oxide and manganese chloride were used for preparing solutions. After standardising these solutions by usual classical methods, these were diluted to give 0.002M solutions.

Sodium hydroxide :

Approximately 0·1M carbonate free sodium hydroxide was prepared and standardised with analytical grade potassium hydrogen phthalate. It was diluted to give 0·02M solution.

Dioxan :

The B.D.H. dioxan was purified by Weissberger's method[8].

Potentiometric method :

The pH titrations of the ligands with standard alkali solution in absence and in the presence of different metal ions were carried out by using Cambridge bench type pH meter which gives values accurate upto 0·02 pH unit. The pH meter was standardised before, and checked after, each titration with buffer solutions of pH 4·00 and 9·27. Electrode system consisted of glass electrode (pH range 1-13) and reference electrode as saturated calomel electrode.

10 ml. of 0·04M ligand solution in 70% v/v dioxan was pipetted in a titrating vessel, requisite amounts of 0·02M nitric acid was added to it in order to lower the pH to about 2. The final ionic concentration was maintained at 0·1M by adding 5 ml. of 1 M KCl. When titrating in presence of metal ions 5 ml. of 2×10^{-3}M metal ion solution was added. The total volume of the contents was made 50 ml. by adding varying amounts of dioxan and distilled water in such proportions that it finally became 70% v/v dioxan-water mixture. It was reproducible with a maximum variation of $\pm0{\cdot}02$ pH unit. The titration curves are shown in Fig. 1.

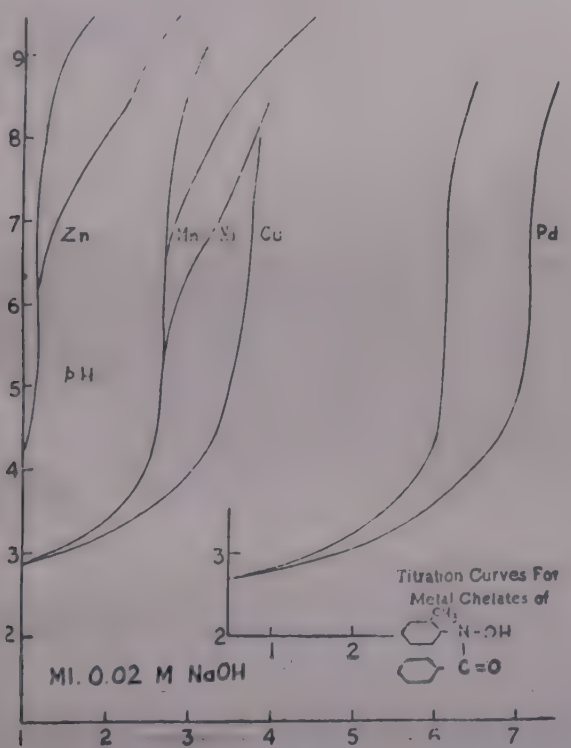

FIG. 1

CALCULATIONS

The horizontal distance between the reference titration curve and the curve obtained in presence of a metal ion gives the amount of metal bound ligand. This value divided by the total metal ion concentration gives $\bar{n}$. In this way the values of $\bar{n}$ at different pH values were calculated

TABLE I

Formation curve data for metal chelates of N-benzoyl-N-o-tolylhydroxylamine

Palladium			Copper		
pH	$\bar{n}$	p [A]	pH	$\bar{n}$	p [A]
2·80	0·2	10·33	2·85	0·3	10·27
2·85	0·4	10·28	2·90	0·4	10·22
3·00	0·6	10·13	3·05	0·5	10·07
3·20	0·8	9·94	3·55	0·8	9·57
3·30	0·9	9·84	3·80	1·0	9·33
3·45	1·0	9·69	3·95	1·2	9·18
3·90	1·2	9·25	4·05	1·3	9·09
4·15	1·3	9·00	4·45	1·5	8·69
4·35	1·5	8·81	4·80	1·6	8·34
4·60	1·7	8·55	5·00	1·7	8·14
4·75	1·8	8·40	5·24	1·9	7·90
5·50	2·0	7·65	5·50	2·0	7·65

TABLE II

Formation curve data for metal chelates of N-benzoyl-N-o-tolylhydroxylamine

Nickle			Zinc		
pH	$\bar{n}$	p [A]	pH	$\bar{n}$	p [A]
5·50	0·1	7·62	6·50	0·1	6·62
5·96	0·3	7·17	6·85	0·3	6·28
6·10	0·4	7·02	7·00	0·4	6·12
6·20	0·5	6·93	7·10	0·5	6·02
6·40	0·6	6·72	7·20	0·6	5·92
6·50	0·7	6·62	7·30	0·7	5·82
6·75	0·9	6·37	7·50	0·9	5·62
6·85	1·0	6·28	7·65	1·1	5·47
7·10	1·2	6·03	7·90	1·3	5·22
7·25	1·3	5·88	8·00	1·4	5·13
7·35	1·4	5·78	8·05	1·5	5·08
7·45	1·5	5·68	8·10	1·6	5·02
7·70	1·7	5·43	8·35	1·8	4·78
7·85	1·8	5·23	8·55	1·9	4·58
8·40	2·0	4·73	8·90	2·0	4·23

TABLE III

Formation curve data for mangnese chelates of N-benzoyl-N-o-tolylhydroxylamine

pH	n	p A
7·25	0·3	5·87
7·40	0·4	5·72
7·70	0·6	5·42
7·90	0·8	5·22
8·25	1·1	4·88
8·50	1·3	4·63
8·65	1·4	4·48
8·90	1·6	4·22
9·15	1·8	3·98
9·30	1·9	3·83
9·45	2·0	3·68

TABLE IV

Stability constants of metal chelates of N-benzoyl-N-o-tolylhydroxylamine

Metal ion		log k_1	log k_2	log k_1k_2
Palladium	...	10·21	8·81	19·02
Copper	...	10·07	8·69	18·76
Nickle	...	6·93	5·68	12·61
Zinc	...	6·02	5·08	11·10
Manganese	...	5·58	4·37	9·95

At any pH, the value of free ligand ion concentration A^- was calculated from the total ligand soncentration HA, and its ionisation constant. This is based on the assumption that the amount of chelating agent over metal ion is so great that the removal of HA by chelation does not cause any significant change in equilibrium :

$$HA \rightleftharpoons H^+ + A^-$$

The pKa value of N-benzoyl-N-o-tolylhydroxylamine was potentiometrically[9] found to be 11·01 in 70% v/v dioxan-water mixture.

In calculating the values for $\bar{n}$ and $[A^-]$, the concentration was corrected for changes in volume, produced by addition of alkali during titrations. This way a series of values for $\bar{n}$ and $[A^-]$, corresponding to different values of pH, was obtained. The results are given in Tables 1-3.

The formation curves of metal chelates of N-benzoyl-N-o-tolylhydroxylamine are shown in Fig. 2.

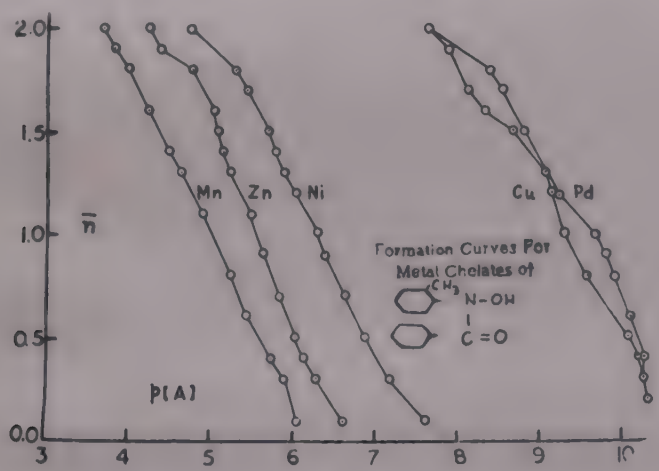

FIG. 2

The values of log k_1 log k_2 and log k_1k_2 for various metal chelates were read directly from the formation curves and are given in Table IV.

DISCUSSION

The consumption of alkali during the course of titration can be due to the ligand, the hydrolysis of metal and the ligand protons liberated in complex formation.

The ligands under study are very weak acid pKa = 11·01. Thus, the ligand protons as such are not in titrable form. Hydrolysis of these metal ions in 70% dioxan-water mixture has been studied and is not likely to interfere in the stability determinations. Moreover, there was no precipitation during the chelation titrations, ruling out the possibility of hydrolysis of these metal ions in presence of large excess of ligands. Thus, the consumption of an excess of alkali in chelation titration over the simple ligand titration is due to the ligand protons liberated during the complex formation. This may be represented as:

$$M^{+2} + HA + OH^- \rightleftharpoons MA^+ + H_2O$$
$$MA^+ + HA + OH^- \rightleftharpoons MA_2 + H_2O$$

The following order of stability of N-benzoyl-N-o-tolylhydroxylamine metal chelates was found. Palladium>Copper>Nickel>Zinc>Manganese.

Summary

Stabilities of palladium, copper, nickel, zinc and manganese chelates of N-benzoyl-N-o-tolylhydroxylamine have been determined in 70% v/v dioxan-water mixture by employing Bjerrum-Calvin pH titration technique. The titration medium was maintained at a constant ionic strength (0·1 M KCl) and at a temperature 25 ± 0·5° log. K, palladium 19·02, copper 18·76, nickel 12.61, zinc 11·10, manganese 9·95.

Acknowledgement

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NOTE ON THE ESTIMATION OF THE CONSTITUENTS OF ANTISPASM TABLET CONTAINING CODEINE PHOSPHATE, AMINOPYRIN, PHENACETIN, PHENOBARBITONE PHENOLPHTHALEIN

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The antispasm tablet with codeine phosphate, Aminopgrin, phenacetin Phenobarbitone, phenolphthalein as constituents poses a problem to analyst because the method of determination by the formation of compound with $Hg\text{-}I_2$ and using the method of phenolphthalein tablet as stated in the British pharmacopoeia, is not satisfactory. The process of extracting the individual constituents by Water-extraction is also futile. The present communication discusses the possibility of estimation using acid and then extraction by suitable organic solvent.

REAGENTS

(1) Stock solution of dilute NH_4OH, 20% NaOH, $\frac{N}{1}$ NaOH

(2) Stock solution of $\frac{N}{1}$ HCl

(3) Stock solutions of $\frac{N}{50}$ HCl, $\frac{N}{50}$ NaOH

(4) Solution of methyl red

(5) Suitable quantity of sodium chloride A.R. and sodium Bicarbonate B.P. is used.

(6) Sufficient solvent Ether B.P., Chloroform B.P., Benzene B.P.

PROCEDURE

The average weight per tablet is determined and the tablets are powdered in a mortar. About 1·5 gm. of the fine powder is weighed accurately and transferred to a separating funnel containing 10 ml. of $\frac{N}{1}$ HCl. The powder is mixed well with the acid in the separator by shaking. The acid solution is extracted with five successive portions of 15 ml of a mixture of 2 volumes of

chloroform and 1 volume of ether. Each extract is washed with the same quantity of 10 ml. of $\frac{N}{10}$ HCl. The ether-chloroform extract contains phenacetin, phenobarbitone and major portion of phenolphthalein. This mixture is retained in a separator (marked A).

To the aqueous liquid (acidic) in the first separator the acid washing of the second separator is added. The combined acid liquid is extracted with five successive portions of 20 ml. of benzene. The aqueous (acid) liquid is retained for codeine phosphate in another separator (marked B). The combined benzene layer is made alkaline to litmus paper with dilute ammonium hydroxide solution. The benzene layer is shaken well and allowed to separate. It is then washed with small quantity of distilled water until free from any pink colour due to the presence of traces of phenolphthalein. The benzene-extract is evaporated in a tared basin on water bath at a temperature not exceeding 80°C. The residue is dried to constant weight at 80°C. The dried residue is weighed and calculated as (C13 H 17 0 N3) Aminopyrin per tablet of average weight.

The alkaline washings are added to the acid liquid retained for codeine phosphate in the separator (marked B). The combined acid liquid is made alkaline with dilute ammonium hydroxide. The alkaloid is extracted with five successive quantities of 25 ml of chloroform. Each portion of chloroform extract is washed with the same 10 ml. of distilled water. The alkaline aqueous liquid and washings are collected for phenolphthalein in a separator (marked C). The combined Chloroform is evaporated in a beaker to almost dryness on a waterbath, 5 ml. of 95% alcohol is added and then evaporated to complete dryness. The residue is dissolved in 10 ml. of $\frac{N}{50}$ HCl. and the excess acid is titrated back with $\frac{N}{50}$ NaOH using methyl red solution as indicator. Each EL of $\frac{N}{50}$ HCl consumed by the alkaloid is equivalent to 0·008128 gm of C 18 O3 N, H_3 P.O. 4, ($\frac{1}{2}$ H_2 0) codeine phosphate.

The content of codeine phosphate per tablet of average weight is calculated.

The either-chloroform mixture retained in the separator (marked 'A') is shaken well with 20 ml of 20% $\frac{W}{V}$ NaOH solution for five minutes. The liquid is allowed to separate. The lower portion of the extract is transferred into another separator containing 1 ML. of $\frac{N}{1}$ 0H and 10 ml. of water. The ether-chloroform mixture is extracted with alkaline liquid. This is then washed with 10 ml. of water. The combined ether-chloroform layer is evaporated in a tared basin on water bath at 100°C to constant weight. The dried mass is weighed and calculated as ($C_{10}H_{13}O_2N$) phenacetin per tablet of average wt. The combined sodium hydroxide liquid and washings retained in a separator (marked D) is saturated with sodium Chloride A R. The mixed liquid is acidified with HCl. The acidic liquid is extracted with five successive portions of 20ml. of solvent ether. The aqueous portion is retained for phenol-

phthalein in a separator (marked E) The ether layer is washed with 10 ml. of distilled water. The ether is evaporated in a tared basin on water bath at 100° C to constant weight. The dried mass is weighed and calculated as ($C_{12}H_{12}N_2O_3$) phenobarbitone per tablet of average weight.

The acidic liquids retained for phenolphthalein in the separator (marked E) and the alkaline liquids with washings retained for the same purpose in the separator (marked C) are mixed in a separator. The whole liquid is first made slightly acidic and then a little sodium bicarbonate is added to make the liquid alkaline to litmus paper. The liquid is extracted with five successive quantities of 15 ml. of a mixture of 2 volumes of solvent ether and 1 volume of chloroform. The combined extracts are washed with a little distilled water. The liquid extracts are evaporated in a tared basin on water bath to constant weight. The dried mass is weighed and calculated as ($C_{20}H_{14}O_4$) phenolphthalein per tablet of average weight.

Discussion

It is seen that there is no method of assay of amidopyrin in Pharmacopoeia of India and the British Pharmacopoeia. Hence, the method discussed here is based on the solubility of aminopyrin in acid and benzene. The present method is very simple and the yield is about 99·0% of the stated amount for the different constituents.

Acknowledgement

Thanks are due to Mr. S. Basu, Deputy Managing Director and Dr. S. N. Ghosh, Chief Chemist, Bengal Chemical, for providing the opportunity to carry out the experiment.

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CATIONIC EQUILIBRIUM WITH REFERENCE TO ALKALISATION OF SOILS

By

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Early studies to explain ion-exchange equilibrium were based on empirical equations that were only modification of either Law of Mass action or Langmuir and Freundlich adsorption isotherms. Kielland (1935)[5] made an improvement by the use of thermodynamic model. Further development as well as complete form was given by Gaines and Thomas (1953)[3]. Guoy's diffuse double layer theory of charged surface and Donnan membrane effects were later put to use in the advancement of knowledge of ion-exchange equilibrium (Helmy 1964,[4] Heald, Frere and Dewit, 1965)[7].

The basic work on ion-exchange of soil had been only with homo-ionic clays. But polyionic systems met with in problems under practical agriculture and Schofields' ratio law has been of immense use in this direction. Wilcox[8] was perhaps the first to bring out the relationship between SAR of irrigation water and equilibrium ESP of soil colloids. Levy *et al.* (1965)[6] made similar studies in Israel.

An attempt based on Guoy-Stern model was made in red and black soils on the assumption that ion-exchange is controlled by Nernst Potential across the diffuse double layer. This model may well serve as a semi-quantitative method for practical utility in as much as swelling pressures and interactions of other ionic species[1] are ignored.

MATERIALS AND METHODS

One typical red soil and another black soil of Coimbatore district having basically different types of clay were taken. The composition of exchangeable ions was determined by conventional methods (Table 1).

TABLE I

The initial analysis of Red and Black soils

	meq 100 g^{-1} soil				E.C. 1 : 2 soil solution mmho cm^{-1}
	C.E.C. meq	E_{Na}	Clay %		
Red soil	16.9	2.61	14.00	36.2	<0.2
Black soil	46.6	6.50	38.40	48.9	<0.2

The water with a total salt content of 2500 ppm with 9·5 SAR was prepared and used in the study ; 5 g of soil was allowed to come in equilibrium with 50 ml water, centrifuged and the solution phase separated for analysis of sodium by Flame Photometer and ca + Mg by Versenate method. The solid phase was washed with 60% alcohol and exchangeable ions determined. The soils were repeatedly treated with saline water five times. The composition of solid and solution phases was determined each time. High saline water was used to facilitate easy separation of phases after equilibrium.

The Nernst Potential E arising from the Diffuse layer acting as Donnan membrane and consequent differences in the distribution of cations on solid phase and in the bulk of solution phase is given by :

$$E = \frac{RT}{nF} \text{ In } \frac{a1}{a2} \qquad (1)$$

where a1 and a2 are the activities of ions at the solution and solid phases respectively, n the valency of ions, T the temperature and R and F, the universal constants.

For different species of ions in equilibrium the potential E will be the same since the system as a whole has attained equilibrium. Considering mainly monovalent and divalent ions,

$$E = \frac{RT}{F} \text{ In } \frac{M1}{M2} = \frac{RT}{2F} \text{ In } \frac{D1}{D2} \qquad (2)$$

M = Monovalent ionic species
D = Divaltnt ionic species.
Subscript 1 = Liquid phase
Subscript 2 = Solid phase

Therefore it follows :

$$\frac{M1}{M2} = \left(\frac{D1}{D2}\right)^{\frac{1}{2}} \qquad (3)$$

Rearranging (3)

$$\frac{M1}{(D1)^{\frac{1}{2}}} = \frac{M2}{(D2)^{\frac{1}{2}}} \qquad (4)$$

In the solution phase $\left(\frac{M1}{D1}\right)^{\frac{1}{2}}$ is called SAR when the monovalent sodium ions are considered. These ratios are usually called "activity ratio" for any particular ionic species.

The 'activityratio' of monovalent to divalent ions, in solution phase as well as solid phase tends to be the same when the equilibrium has been attained. This is true for homogenous system where concentrations will be expressed in the same units. But in heterogenous systems, the concentrations will be in

different units in the two phases. Hence, it becomes necessary to introduce a constant to allow for the differences in expression of concentrations. The equation (4) will then be modified as:

$$\frac{M1^{\frac{1}{2}}}{(D1)^{\frac{1}{2}}} = K \frac{M2^{\frac{1}{2}}}{(D2)^{\frac{1}{2}}} \qquad (5)$$

A rigorous theoretical approach naturally entails consideration of activities at the solid surface involving difficulties in the choice of standard state and the associated calculations. A simplification can, however, be made in the context of dynamic nature of equilibrium where the activity coefficients both at the solid and the solution phases may be assumed to be the same. Even this necessitates calculations the degree of accuracy of which offers little scope in practical application.

Therefore, the following expression of concentrations were adopted for arriving at the constant K.

Concentration in solution phase a mm litre^{-1}

Activity coefficient: Debye Huckel approximation formula.

Concentration at solid phase mM 100 g^{-1} clay

Results and Discussions

With the constant value of K obtained (Table II) the equilibrium of the saline soil may be said to have been achieved even at the first leaching.

TABLE II

Ratio of SAR liquid phase (L) to solid phase (S) with conc. and activity terms

Soil group	$SAR_{(L)}/SAR_{(S)}$						$^{a}SAR_{(L)}/^{a}SAR_{(S)}$					
	Leaching						Leaching					
	1	2	3	4	5	AV	1	2	3	4	5	AV
Red	3·69	3·47	3·64	3·13	3·73	3·63	3·63	4·79	4·36	4·64	4·74	4·64
Black	2·34	2·42	2·52	2·39	2·31	2·41	3·00	3·04	3·27	3·08	3·07	3·09

Solid phase adsorption ratio and solution phase activity are found positively associated (Fig. 1). Small differences were however noticed in the activity ratio of the solution phases which may be ascribed to dissolution of reserve calcium carbonate by sodium chloride. By employing the term mole fraction at the solid phase the concentration may be rendered dimensionless and this again has the same positive association with a_{SAR} (Table III-Fig. 2).

TABLE III

Ratio of a_{SAR} liquid phase to mole fraction of sodium (x_{Na}) at solid phase

Soil group	$^{a}SAR_{(L)}$			$^{x}Na_{(S)}$		
	1	2	3	4	5	AV
Red	0·32	0·32	0·32	0·34	0·34	0·33
Black	0·29	0·31	0·33	0·32	0·31	0·31

The results at best may serve as a semi-quantitative measure and much weight on the thermo-dynamical aspects cannot be attached to the values. However, the constant K may be viewed from the Law of Mass action, as follows:

$$MS + \tfrac{1}{2}DL \rightleftharpoons ML + \tfrac{1}{2}Ds$$

(L = solution phase ; S = solid phase)

$$K = \frac{(ML)\quad (DS)^{\frac{1}{2}}}{(MS)\quad (DL)^{\frac{1}{2}}}$$

Gilbert and Laudelout (1965)[2] obtained a similar constant called selectivity coefficient in studying the exchange properties of hydrogen clay. Heald *et al.* (1965)[7] used the term formation constant in their study of 'ion pair' formation based on Gouy-Stern models. Despite the limitation imposed by the simplicity of the model and the neglect of swelling pressures as also the effect of other ionic species, the constant K nevertheless helps to indicate the effect of solution phase on the exchangeable sodium, a factor important in the study of alkalisation of soils. The value of K can therefore be used to compare the ease with which alkalisation or de-alkalisation may occur in different soils. The free energy change accompanying the reaction as represented in the above equation is calculated and given below for soil types studied.

TABLE IV

Free energy changes in the alkalisation process

Soil group	Free energy change in calories*	
	De-alkalisation	Alkalisation
Red	−921	+921
Black	−770	+770

The free energy change so obtained from K indicates the ease with which de-alkalisation can taken place from red soils compared to that of black soils which are in agreement with general experience. The positive value of free energy change for alkalisation indicates the reluctance of sodium to enter the solid-phase which in other words means the weak adsorption of monovalent ions.

Summary and Conclusion

Based on Guoy-Stern and also Donnan theories, a simple model was evolved and a relationship is worked out for the distribution of mono and divalent ionic species in the solid and solution phases. The effect of swelling pressures, the presence of other ionic species and solid phase activity have been ignored. Nevertheless, they indicate great possibilities and find application in the

* The quantities referred to are in calories for exchange of one mM Na from 100 g clay by half mM of divalent ions in one litre of solution and *vice versa*.

alkalization and de-alkalisation of soils. Factors such as constancy of K serve to predict exchangeable Na for values of SAR. The simplicity of the model may find application as a semiquantitative method were rigid theoretical assumptions do not have a play.

Acknowledgment

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IMPEDANCE TITRATION : POTASSIUM IODIDE Vs. POTASSIUM PERMANGANATE

By

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It has been reported that when a constant alternating current is applied between two platinum electrodes (one of them polarisable electrode and the other a non-polarisable electrode) immersed in a titrate, the impedance of the system changes during the course of the titration, the equivalence point being indicated by a sudden change in the impedance, which is manifested by a large change in the alternating voltage across the electrodes. This titration has been termed as "polarisation titration"[1] or more aptly as "impedance titration"[2, 3, 4].

Hendrixson[5, 7] showed that the potentiometric titration of iodides with potassium permanganate gave very accurate results and this was confirmed by Kolthoff[6, 7]. Rao[8] reported that KI-$KMnO_4$ system responded to the techniques of impedance and redoxo-kinetic titrations. In this paper, the effect of current density and acid concentration on the accuracy of the equivalence point has been studied and the results are compared with those obtained potentiometrically. The applicability of the impedance titration technique for different dilutions has also been studied.

EXPERIMENTAL

The circuit diagram is given in Fig. 1. A constant current was maintained from the AC source with the help of the variable resistances. The alternating current was measured by passing it through a non-inductive standard resistance (100Ω) and measuring the voltage drop across the resistance with the help of the vaccum tube voltmeter (Philips GM6012 with frequency response of 2 c/s to 1 Mc/s). The change in the impedance of the system during the course of the titration was followed by a change in the voltage across the electrodes using the VTVM.

A 0·45 mm. dia × 0·7 cm. long platinum wire fused to a glass tube to which electrical contact was given through mercury and a platinum gauze (1·2 cm. dia. × 4·5 cm. ht.) acted as polarisable and non-polarisable electrodes respectively. The gauze electrode just surrounded the experimental micro-electrode thereby minimizing the iR drop across it. It was found that neither special

pretreatment of the electrodes nor the removal of the dissolved oxygen was necessary during this titration. The titration was carried out at room temperature.

Analytical grade chemicals were used for the preparation of the solution. The composition of the solutions used in different experiments are given below :

Titrate :

1 a 20 ml. of 0·09804N potassium iodide + 15 ml. of 2N sulphuric acid + 50 ml. of distilled water.

b 20 ml. of 0·09804N potassium iodide + 15 ml of IN sulphuric acid + 50 ml. of distilled water.

c 20 ml. of 0· 09804N potassium iodide + 15 ml of 4N sulphuric acid + 50 ml. of distilled water.

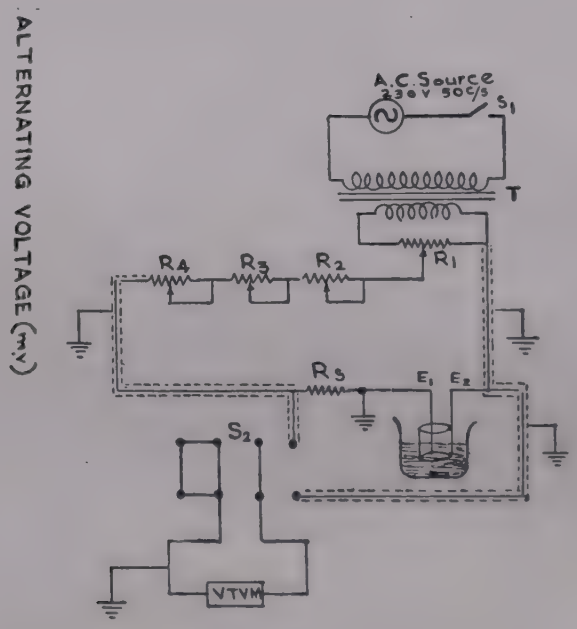

FIG. 1

CIRCUIT DIAGRAM
T=Step-down transformer (230 V/6·3V)
R_1, R_2, R_3 and R_4=Potentiometers (10K, 100K, 10K and 1K respectively)
R_5=Standard resistance (100 Ω) (Non-inductive)
S_1=Switch; S_2=DPDT Switch
E_1=Platinum microelectrode; E_2=Platinum gauze electrode

Titrant :

0·120N potassium permanganate.

Titrate :

2 a 20 ml. of 0·009705N potassium iodide + 15 ml. of 2N sulphuric acid + 50 ml. of distilled water.

b 20 ml. of 0·0009705N potassium iodide + 15 ml. of 2N sulphuric acid + 50 ml. of distilled water.

Titrant :

0·0120N potassium permanganate.

After each addition, the solution was well stirred by a magnetic stirrer, and the alternating voltage across the electrodes was measured using the VTVM Since the impedance is directly related to the alternating voltage at a constant alternating current, the graphs are drawn taking the alternating voltage across the cell (VTVM reading) as ordinate and the volume of the titrant as absciassae.

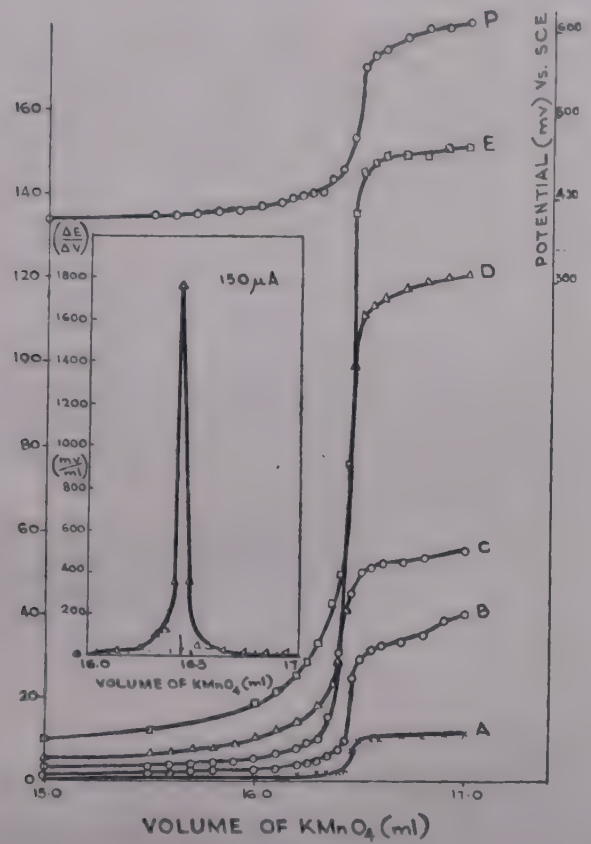

FIG. 2

Titration of 20 ml. of 0·09804N KI vs 0.120N $KMnO_4$ at various alternating currents
P=Potentiometric titration
A to E=Impedance titration
A=15μA; B40μA;
C=74μA; D=μA;
E=300μA

RESULTS AND DISCUSSION

Typical plots of the alternating voltage for impedance titration and the redox potential for potentiometric titration against the volume of titrant during the course of various titrations are presented in Figs. 2 to 5.

Effect of current density :

With the increase in the alternating current (i.e. at various current densities) the alternating voltage across the cell also increases. There is a large variation at the equivalence point. There is a striking coincidence in the values of end-points obtained by the two methods. Inset in Fig. 2 shows the derivative for AC 150 μA (r.m.s.). From the figure, it is seen that a sharp jump is

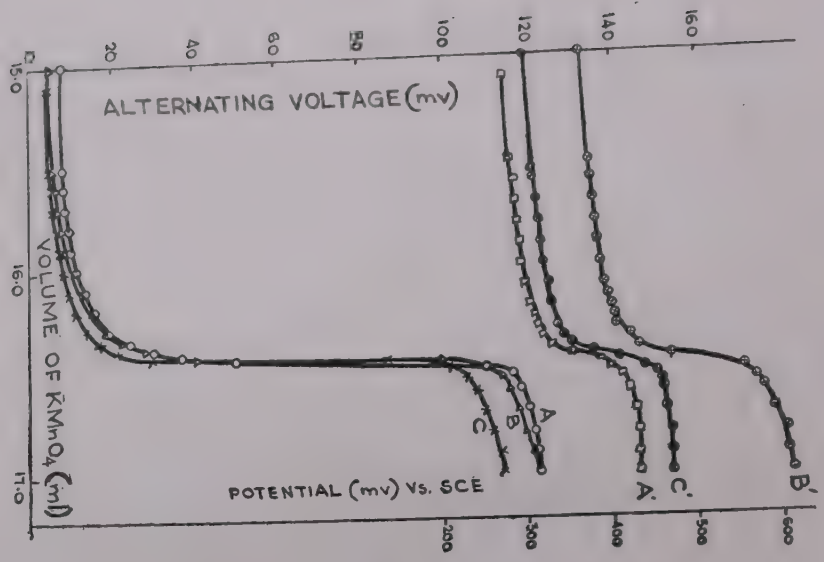

FIG. 3

Titration of 20 ml. of 0·09804N KI vs 0·120N $KMnO_4$ at different acid concentrations
A, B, C: Impedance titration
A′, B′, C′: Potentiometric titrations
A, A′=1N Sulphuric acid; B, B′=2N sulphuric acid; C, C′=4N Sulphuric acid

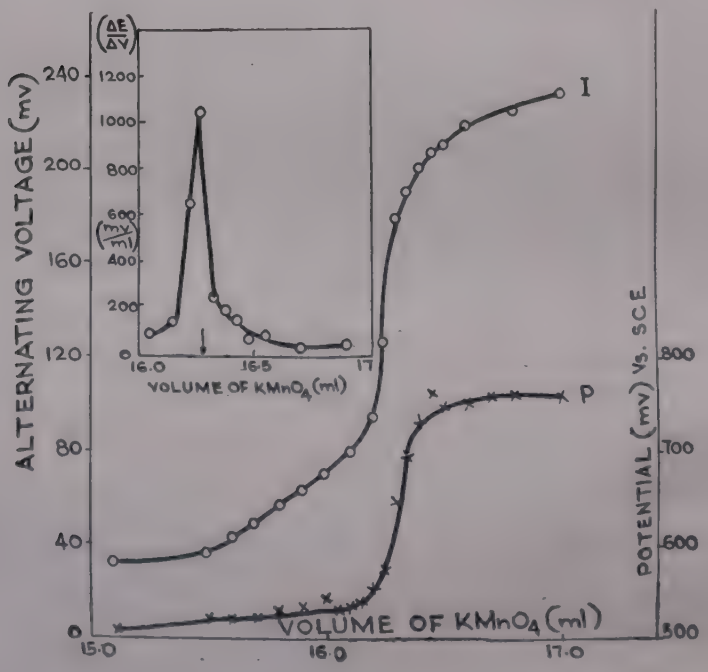

FIG. 4

Titration of 20 ml. of 0·009705N KI vs 0·0120N $KMnO_4$
P=Potentiometric titration
I=Impedance titration

obtained when the AC is 150 μA (current density 1·5 mA/cm.^{2}) and above this value $\frac{\Delta E}{\Delta V}$ (not shown in the figure) has not changed very much. Hence, the subsequent experiments were carried out using the constant current of 150 μA(r.m.s.).

Effect of acid concentration :

From the figure 3, it is clear that the acid concentration has no effect on the impedance titration technique. However, in the potentiometric titration the jump in the potentials at the end-point for the same volume of 1N and 4N sulphuric acid is less than for 2N sulphric acid. According to Kolthoff[7] the liquid must contain enough sulphuric acid to make it 0·2N of the titration of this system potentiometrically and hence it appears that the acid concentration is critical for the potentiometric titration.

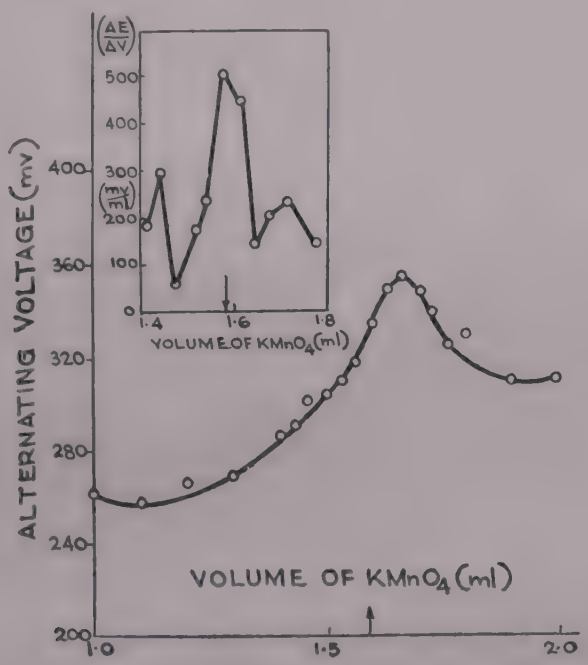

FIG. 5
Titration of 0·0009705N KI vs 0·0120N $KMnO_4$
(Impedance titration)

Effect of dilution :

When the titration is carried out using the concentration of titrate and titrant to approximately N/100 (0·009705N) of KI with 0·0120N $KMnO_4$) both the methods give sharp end-points (Fig. 4). However, when the concentration of titrate and titrant is reduced to approximately N/1000, both the methods are unsuccessful. At the same time, as the impedance titration is suitable for the titration of approximately 0·001N KI (0·0009705N) with approximately 0·01N $KMnO_4$ (Fig. 5); the potentiometric titration is unsuccessful.

In all the titrations, it is observed that the potential assumes steady value more slowly just at the equivalence point. After all the iodine has been liberated, the addition of excess of permanganate lowers the potential, which has been attributed to the oxidation of iodine to iodate by excess permanganate[7].

SUMMARY

1. The impedance titration technique was used for KI-$KMnO_4$ system and their results were compared with those obtained potentiometrically.

2. It was found that the sharp jump was obtained at a current density of 1·5 mA/cm^2.

3. The variation of concentration of the supporting electrolyte (sulphuric acid) had no effect on the impedance titration technique.

4. The impedance titration technique was suitable for the titration of 0·001N KI with 0·01N $KMNO_4$, whereas the same was unsuccessful by the conventional potentiometric method.

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J. Inst. Chemists (India), Vol. XL, March, 1968

A NOTE ON PHYTOCHEMICAL INVESTIGATION OF THE OIL OF TERMINALIA BELERICA

By

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The oil extracted from kernels of Terminalia Belerica Roxb (N.O. Camberetacae) is used as substitute for Ghee.[1,2] The physical constants of the oil have already been reported[3]. The phytochemical investigation of the oil in respect of its amino acids, sterols and vitamin contents are presented in this paper.

Experimental

The kernels of the fruits of Terminalia belerica, were dried at room temperature and coarsely powdered. The coarse powder (450 g) was extracted with petroleum ether (60-80°) for 48 hours. A yellow coloured oil (229 g) was left on recovering the solvent from petroleum ether extract.

AMINO ACIDS—The amino acids were isolated by treating 20 ml of the oil with 30 ml of water and 7 ml of 2N HCl. The emulsion formed on shaking the mixture vigorously for four hours was transferred in a separating funnel and left overnight. The clear aqueous layer thus separated on evaporation over water-bath left a residue of amino acids as hydrochloride. The residue was dissolved in 1 ml of water saturated with n-butanol. The circular paper chromatography of this solution using butanol-acetic acid-water (4 : 1 : 1) as solvent system resulted in the separation of amino acids. The chromatogram was developed with ninhydrin (0·1% solution in n-butanol). The developed chromatogram was compared with an authentic specimens of amino acids. This comparison confirmed the presence of lysine, glycine and alanine is the oil.

STEROLS—The presence of sterols in the oil was indicated by Lieberman's reaction. The sterols were isolated by refluxing 40 g of oil with 10% alcoholic potassium hydroxide for $2\frac{1}{2}$ hours and the mixture was left overnight. The mixture was stirred after adding 70 ml of water and extracted with successive quantities of ether. The residue obtained after removal of ether was treated with petroleum ether (60-80°). A semi-crystalline residue (1·6 g) was obtained on removal of petroleum ether. The semi-crystalline residue was then run through alumina column using benzene and petroleum ether as elutants. The crystalline residue (113-115°) thus obtained gave colour reactions with the following reagents indicative of sterols.

100 mg of the residue was dissolved in 2 ml of chloroform, and a few drops of acetic anhydride and 1 to 2 drops of concentrated sulphuric acid were added. An orange red colouration was formed. The product also gave reddish violet colouration when treated with 9 parts of trichloroacetic acid.

VITAMINS—The Carr-Price[4] method indicated absence of vitamin A. The analysis for vitamin D content was not possible in this laboratory.

Acknowledgement

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J. Inst. Chemists (India), Vol. XL, March, 1968

A USEFUL MODIFICATION OF THE FISKE-SUBBAROW METHOD FOR THE ESTIMATION OF SERUM INORGANIC PHOSPHATE

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Fiske and Subbarow[1] introduced the method of estimation of inorganic phosphate by a colour reaction between 1 : 2 : 4-amino naphthol sulphonic acid (ANSA) and phosphomolybdic acid formed by the phosphate with a molybdate reagent. This method is very widely used for estimation of inorganic phosphate in biological fluids including blood, urine, etc.

This reaction, when routinely applied for the determination of inorganic phosphate in human serum, however, presented certain difficulties. The amino naphthol sulphonic acid reagent, although claimed by the authors to be stable for about 4 weeks, deteriorates much earlier. Use of fresh reagent everyday becomes difficult due to the complicated method of its preparation requiring both sodium sulphite and sodium bisulphite. The coloured solution obtained at the final stage is not sufficiently stable for taking colorimetric reading at ease. The rate of increase of the intensity of the colour is also dependent on the room temperature thus making it difficult to prescribe for the whole year a fixed interval of time after which the reading should be taken. There are several modifications of this method in the literature all of which aim at a more suitable reducing agent than ANSA. For instance, Kuttner and Cohen[2] as well as Shinowara, Jones and Reinhart[3] used stannous chloride while Lowry and Lopez[4] and Nath and Chatterji[5] used ascorbic acid instead of ANSA. But in these methods also the colour intensity could not be stabilized. Nath and Chatterji[5] and Nath and Ghosh[6] introduced the method of taking colorimetric readings at definite intervals of time and then extrapolated the results to get the reading at zero time which was used for calculation. This can surely eliminate the error and disadvantage caused by the gradual change in the intensity of the colour, but obviously such a procedure is unsuitable for a routine laboratory.

We, therefore, decided to reinvestigate the method of Fiske and Subbarow with a view to making it more suitable for routine estimation of serum inorganic phosphate. The result of this investigation is presented in this paper.

Experiments and Results

(A) *Reagents :*

1. *Working standard solution of phosphate* :—A stock standard solution of monopotassium phosphate was prepared by dissolving 0.351 g. of the dry

substance in water and 10 ml. of 10 N sulphuric acid added to it, the volume being made upto 1000 ml. with water. Whenever necessary, a working standard solution was prepared fresh by diluting 1 ml. of the stock standard to 10 ml. with 10% trichloracetic acid. It contains 0.008 mg. phosphorus per ml.

2. *Molybdate II reagent :* Reagent grade ammonium molybdate (25 g.) was dissolved in 1000 ml. of water containing 300 ml. of 10 N sulphuric acid according to the procedure of Fiske and Subbarow.[1]

3. *Amino naphthol sulphonic acid (ANSA) reagent :* An accurately weighed quantity (30 mg.) of 1 : 2 : 4-amino naphthol sulphonic acid was dissolved in 5 ml. of sodium sulphite solution of known concentration ; 5%, 10%, 15% and 20% solutions of anhydrous sodium sulphite were used. No sodium bisulphite was required. Fresh solutions of ANSA were always employed.

4. *Trichloracetic acid :* A 10% solution was needed.

(B) *General procedure for estimation :*

Serum prepared from human blood was made protein free by mixing 2 ml. with 8 ml. of trichloracetic acid. The precipitated protein was filtered off. Then, reagents and solutions were added in the following way in three test tubes marked 'Blank', 'Standard' and 'Unknown'.

	Blank	Standard	Unknown
10% trichloracetic acid	5 ml.	—	—
Working standard of phosphate	—	5 ml.	—
Protein-free filtrate of serum	—	—	5 ml.
Molybdate II reagent	1 ml.	1 ml.	1 ml.
Distilled water	3·6 ml.	3·6 ml.	3·6 ml.
ANSA reagent	0·4 ml.	0·4 ml.	0·4 ml.

Ten minutes' time was allowed after mixing and then the bluish green colour was read in a photoelectric colorimeter at 630 mμ (red filter). The result in terms of mg. of phosphorus per 100 ml of serum was obtained from the formula :

$\frac{U}{S} \times 4$ where U and S represented the reading (optical density) of the *unkuown* and of the *standard* respectively.

(C) *Determination of minimum concentration of sodium sulphite :*

Four different experiments were carried out with working standard solution of phosphate using ANSA in 5%, 10%, 15% and 20% solution of sodium sulphite respectively. With more dilute sodium sulphite no clear solution of ANSA could be obtained. Readings were taken in a Bharat-Adco photoelectric colorimeter and the optical density was found to be $4{\cdot}5 \pm 0{\cdot}05$ in all of the four experiments. This clearly pointed out that 5% solution of sodium sulphite was enough for the purpose and 20% solution as recommended by Fiske and Subbarow was not necessary. It was therefore, decided to use 5% solution for the present method.

(D) *Stability of the colour with time :*

An experiment was arranged with the working standard solution of phosphate using ANSA in 5% sodium sulphite. Readings were taken at definite intervals upto 30 minutes in a Bharat-Adco photoelectric colorimeter. The results, presented in Table I below, show that the intesity of colour is steady during this period. It was decided on these findings to fix 10 minutes time interval for taking reading after the addition of ANSA regent in this method.

TABLE I

Optical density at different intervals

Time in min.	2	4	6	8	10	12	15	20	25	30
Reading	4·5	4·5	4·6	4·65	4·6	4·7	4·7	4·6	4·6	4·7

(E) *Agreement with Beer-Lambert law :*

Working standard solutions of phosphate in 10% trichloracetic acid were prepared in different concentrations ranging from 0·32 mg.% to 0·96 mg.% phosphorus which covers the usual range in the protein-free filtrate of human serum. Experiments were carried out with each of them using ANSA in 5% sodium sulphite. The results are presented in Table II.

TABLE II

Optical density at different concentrations of phosphate

mg. % P	0·32	0·48	0·64	0·80	0·96
O.D.	1·90	2·90	3·75	4·55	5·35

The plot of optical density against concentration was linear showing that Beer-Lambert law was followed within the limits of concentration used.

(F) *Comparison of the results by the present method and the Fiske-Subbarow method :*

Fifteen specimens of human sera were analysed for their inorganic phosphate content using ANSA prepared by the present method as well as by the method of Fiske and Subbarow[1]. Each pair of experiments was carried out simultaneously. The results are given in Table III.

TABLE III

Serum inorganic phosphate by present method and Fiske-Subbarow method

Serial No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
mg. % P present method	6·7	3·7	3·4	2·8	2·1	4·4	4·0	4·2	3·9	4·4	3·7	3·8	3·7	4·7	2·6
mg. % P F.S. method	6·5	3·7	3·5	2·7	2·0	4·4	3·9	4·2	3·8	4·2	3·7	3·6	3·6	4·4	2·6

Statistical analysis done to compare the two sets of results showed that the difference was not significant.

Conclusion

The findings presented above led to the conclusion that the present method using ANSA in 5% sodium sulphite only can give the result of analysis of serum inorganic phosphate as accurately as the original method of Fiske and Subbarow. It has the following additional advantages:

(1) Sodium bisulphite is not required for preparing ANSA solution.

(2) Sodium sulphite required is of much smaller concentration.

(3) The method of preparation of ANSA reagent is more easy.

(4) The intensity of colour of the final solution is more stable and the colorimetric reading can be taken at ease.

(5) It agrees with Beer-Lambert law correctly within the limits of concentration of phosphate selected.

The method has been found to be more advantageous for a routine laboratory.

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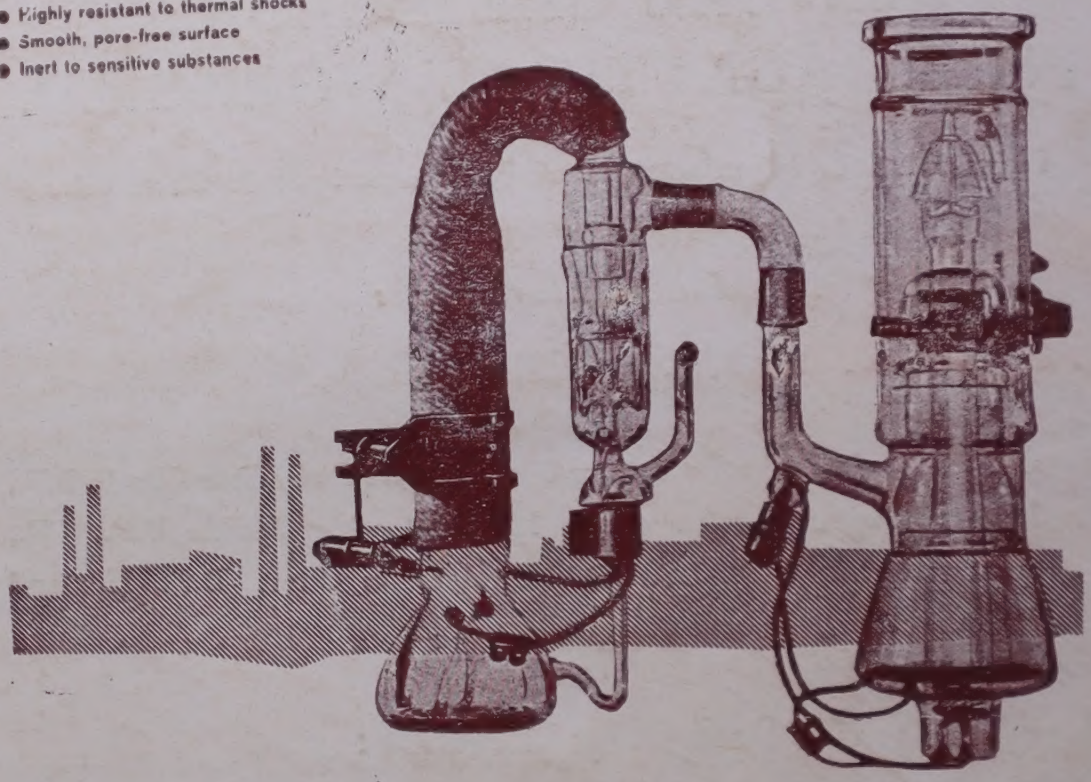

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